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Research Note

Updated distribution records for *Anopheles vagus* (Diptera: Culicidae) in the Republic of Philippines, and considerations regarding its secondary vector roles in Southeast Asia

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Abstract. Distribution records for *Anopheles (Cellia) vagus* in the Republic of the Philippines were updated, including recent collection and museum records from Luzon and Visayas Provinces. Larval habitats (e.g. rice paddies, irrigation and drainage ditches), associated species, and the vector potential of this species were also noted.

Anopheles vagus Doenitz, a member of the Pyretophorus Series, subgenus *Cellia*, is widely distributed in Asia, particularly Bangladesh, Cambodia (Kampuchea), China (including Hong Kong), India, Indonesia, Laos, Malaysia, Mariana Islands, Myanmar (Burma), Nepal, Philippines, Sri Lanka, Thailand and Vietnam (Christophers, 1933; Reid, 1968; Knight & Stone 1977; Ward, 1984; WRBU 2010). Regarding records from the Mariana Islands, Darsie & Cagampang-Ramos (1971a) and Ward (1984) recorded *An. vagus*, *Anopheles indefinitus* (Ludlow), and *Anopheles subpictus* Grassi, on Guam, *An. indefinitus* is recognized from Saipan (Pratt & Siren, 1971, Savage *et al*., 1993), and *An. indefinitus* and *An. subpictus* are recognized on Tinian (Valder *et al*., 1976). *Anopheles vagus* was originally described by Doenitz (1902), from a female collected from Fort de Kock, [West Coast], Sumatra, a male from Banjoe-Biroe, Java, and other specimens from different Indonesian localities (Ceram, Borneo, Lombok, New Guinea, Pulu Raja). Knight & Stone (1977) reported that the type specimens are deposited at the Zoologisches Museum (ZM) des Humboldt Universitaet, Berlin, Germany. Recently, Dr. Joachim Ziegler (personal communication, 30 August 2010 with LMR) noted that *An. vagus* specimens at the ZM comprised two pinned specimens (one female and one male without head) and eight mounted specimens on three slides (six males and two females). All specimens were labeled as "paratypes design. F. Peus", with locality written as "Padang", "Bunjol", or "Batavia" not "Fort de Kock" (= Bukittinggi, 90 km from Padang). Therefore, the holotype of this species is missing or unknown.

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14. ABSTRACT
Distribution records for Anopheles (Cellia) vagus in the Republic of the Philippines were updated, including recent collection and museum records from Luzon and Visayas Provinces. Larval habitats (e.g. rice paddies, irrigation and drainage ditches) associated species, and the vector potential of this species were also noted.

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Anopheles vagus variety *limosus* King (1932) was described from Rizal, Luzon Island, Philippines, and was elevated to subspecies by Colless (1948). This subspecies of *An. vagus* was the only representative of this species recognized in the Philippines prior to 1971. The type of *An. vagus limosus* is deposited in the U.S. National Museum of Natural History (USNMNH), Smithsonian Institution, Suitland, Maryland. As late as Baisas & Dowell (1967) and Cagampang-Ramos & Darsie (1970) *An. vagus vagus* was not recognized in the Philippines, and Baisas (1974) did not record this subspecies from Subic Bay Naval Base, Luzon. However, three years prior to Baisas (1974), Darsie & Cagampang-Ramos (1971b) provided the first records of *An. vagus vagus* in the Philippines based on specimens collected and reared from Luzon and Mindanao islands. Shortly thereafter Ramalingam (1974) elevated subspecies *An. vagus limosus* to species status when he collected both subspecies at the same locality in Sabah, Malaysia. In the current classification of *Anopheles*, *An. vagus* and *An. limosus* are treated as separate species (Harbach, 2004; WRBU 2010).

In this study we examined specimens collected by LMR from the Philippines together with existing specimens that are deposited in the USNMNH to better understand the distribution of *An. vagus*. Also, we have accrued additional references indicating that, under certain circumstances, *An. vagus* should be considered a secondary vector of two or more pathogens that impact human health in Southeast Asia.

Specimen collection and morphological identification

About 21 *An. vagus* larvae were collected from three habitats (rice fields, irrigation ditches and drainage ditches) in Laguna Province in July 2002 using a plastic dipper (5 cm ht, 13 cm diam; Bioquip, Rancho Dominguez, CA). Collected larvae were placed in plastic Whirl-Pak® bags (118 ml, 8 x 18 cm) (BioQuip, Rancho Dominguez, CA) filled approximately 1/2 full with water from the collection site. The Whirl-Pak® was then tightly closed to retain air, placed in a cooler, and brought to the building (temporary laboratory) where most larvae were individually link-reared to adult stage, as morphological voucher specimens for this work. Eclosed adults were pinned on paper points, each given a unique collection number, and identified using diagnostic morphological characters (Reid 1968, Cagampang-Ramos & Darsie, 1970). Voucher specimens and collection records were deposited in the USNMNH.

Molecular identification

DNA was isolated from individual adults (1 or 2 legs per adult) by phenol-chloroform extraction, and direct sequencing was carried out as described by Wilkerson *et al.* (2003). The rDNA ITS2 was amplified, and polymerase chain reaction (PCR) products were directly sequenced using Big Dye 3.0 (Applied Biosystems Inc. – ABI, Foster, CA) with an ABI 3100 sequencer (ABI). The sequence was then edited and analyzed using Sequencher (v 4.8, AB). The ITS2 sequences of *An. vagus* (FJ457631.1, FJ654648.1, EU919718) listed in the GenBank (NCBI 2010) were used to compare and confirm the sequences resulting from the analysis of field collected specimens in this survey.

Distribution

Laguna Province: Calauan (14.150°N, 121.316°E), 3 females (F), reared from larvae collected from rice field, 22 July 2002, coll. no. PH 3-2, 3-4, 3-100 [associated with larvae of *Anopheles (Cel.) tessellatus* Theobald and *Aedes (Aedimorphus) caecus* (Theobald)]; 1 male (M), reared from a larva collected from rice field, 22 July 2002, coll. no. PH 4-27 [associated with larvae of *An. tessellatus*, *Aedes caecus*, *Culex (Culex) tritaeniorhynchus* Giles]; 6 F, 3 M, reared from larvae collected from drainage ditch, 29 July 2002, coll. no. PH 9-5, 9-7, 9-9, 9-12, 9-14, 9-101, 9-102, 9-103, 9-104 [associated with larvae of *Cx. tritaeniorhynchus* and *Lutzia (Metalutzia) fuscana* (Wiedemann)]; Siniloan (14.417°N,

121.450°E), 5 F, 3 M, reared from larvae collected from irrigation ditches, 24 July 2002, coll. no. PH 6-1, 6-1A, 6-2, 6-2A, 6-8, 6-9, 6-10, 6-107 [associated with *Aedes (Neomelaniconion) lineatopennis* (Ludlow) and *Cx. tritaeniorhynchus*]. Additional specimens of *An. vagus* from the USNMNH were examined and recorded. These include the following: *Cebu Province* (?): T. Manga, 12 June 1933, 10 F, 2 M, coll. F. H. S./W. V. King, WRBU Acc. no. 657; *Lanao Del Sur Province*: Masia, Talagian, 3 March 1970, 10 F, coll. A. C. Ramos; *Samar Province:* Osmena, 1 F, coll. L. E. Rozeboom, WRBU Acc. no. 1354; *Quezon Province*: Taiaong, Lalig, 15 June 1970, 10 F, coll. A. C. Ramos. Darsie & Cagampang-Ramos (1971b) also reported the distribution of this species in the Philippines, including Bulacan Province (San Jose del Monte), Laguna Province (Calauan, Majayjay), Quezon Province (Tiaong), Lanao del Norte Province (Kolambugan, Tankal), Lanao del Sur Province (Balindong, Madamba, Marawi, Masiu), and South Cotabato Province (Maitum). Mogi *et al.* (1984) identified and studied *An. vagus* specimens collected in Iguig, Cagayan Province, while Wooster & Rivera (1985) reported them from Mindoro Occidental Province.

Habitat information

The larval habitats in Laguna Province where *An. vagus* larvae were found during this study included: rice fields, irrigation ditches and drainage ditches with slow flowing water, having pH ranging from 6.79 - 7.62 (average pH 7.10, n = 3); conductivity 0.13 - 0.24 uS (average 0.26 uS, n = 3); temperature, 27.40 - 27.70°C (average 27.97°C, n = 3). Reid (1968) noted that larvae of this species are typically found in open muddy pools and in hoof marks, ditches, often in foul water and sometimes in brackish water.

Vector potential

For many years *An. vagus* was not considered a vector of human malaria parasites (Christophers, 1933; Reid, 1968; Ramachandra Rao, 1984). This status was based primarily on thousands of specimens in many blood feeding studies indicating that throughout Southeast Asia *An. vagus* fed primarily (over 90%) on cows and water buffalos and was usually ranked the least attracted to humans of all the *Anopheles* tested (Reid, 1961, 1968; Bruce-Chwatt *et al.*, 1966; Ramachandra Rao, 1984). However, over time evidence has accrued indicating this species may serve as a secondary malaria vector under unusual circumstances that include dense concentrations of humans in association with low numbers or absence of bovids and/or primates (Baker *et al.*, 1987; Maheswary *et al.*, 1994; Amerasinghe *et al.*, 1999; Prakash *et al.*, 2004). These areas included Thailand, Kampuchea, Bangladesh, Sri Lanka, and Assam State (India), respectively. More recently, Verhaeghen *et al.* (2010) considered *An. vagus* a potential malaria vector in the Mekong Region (Kampuchea, Laos, and Vietnam) during their assessment of vector resistance against insecticides, while Manguin *et al.* (2008a) noted that it is a confirmed or secondary vector of malaria in East Timor. The findings in these publications usually occurred when well recognized vectors were uncommon or absent. Such different findings led Manguin *et al.* (2008a) to list *An. vagus* as a secondary malaria vector, but in Manguin *et al.* (2008b) it is not listed as a major malaria vector. Experimental infectivity studies have yielded contradictory results. Tran-Thi-Minh-Phuong *et al.* (1972) found that a brackish water strain of *An. vagus* in Vietnam was not able to develop *Plasmodium falciparum* (Welch), yet Somboon *et al.* (1994) in northwestern Thailand found a fresh water strain susceptible to both *P. falciparum* and *P. vivax* (confirmed by gland dissections and ELISA). All of the variable vector data about *An. vagus* may reflect different strains or even sibling species with different ecological requirements and geographical distributions. Baimai *et al.* (1996) reported two karyotypic forms (A and B) of *An. vagus* in Thailand. Currently, it is not known if these forms represent

intraspecies or interspecies entities. In the Republic of Philippines it has not been reported as a vector of malaria, possibly because of conflicting distribution records and the misidentification of specimens due to confusion with *An. limosus*.

Anopheles vagus is also capable of serving as a vector of other parasites to humans. On Flores Island, Indonesia, this species was found susceptible to infections with the filarial parasite, *Wuchereria bancrofti* (Cobbold) (Atmosoedjono *et al.*, 1977), and was confirmed as a secondary vector of this parasite on that island (Lee *et al.*, 1983). Harinasuta *et al.* (1970) found *An. vagus* females with larvae of *W. bancrofti* (0.51% infection rate, larval stages I and II) and *Dirofilaria* spp. (0.51% infective rate, larval stage III) in Thailand. Manguin *et al.* (2010) listed *An. vagus* as

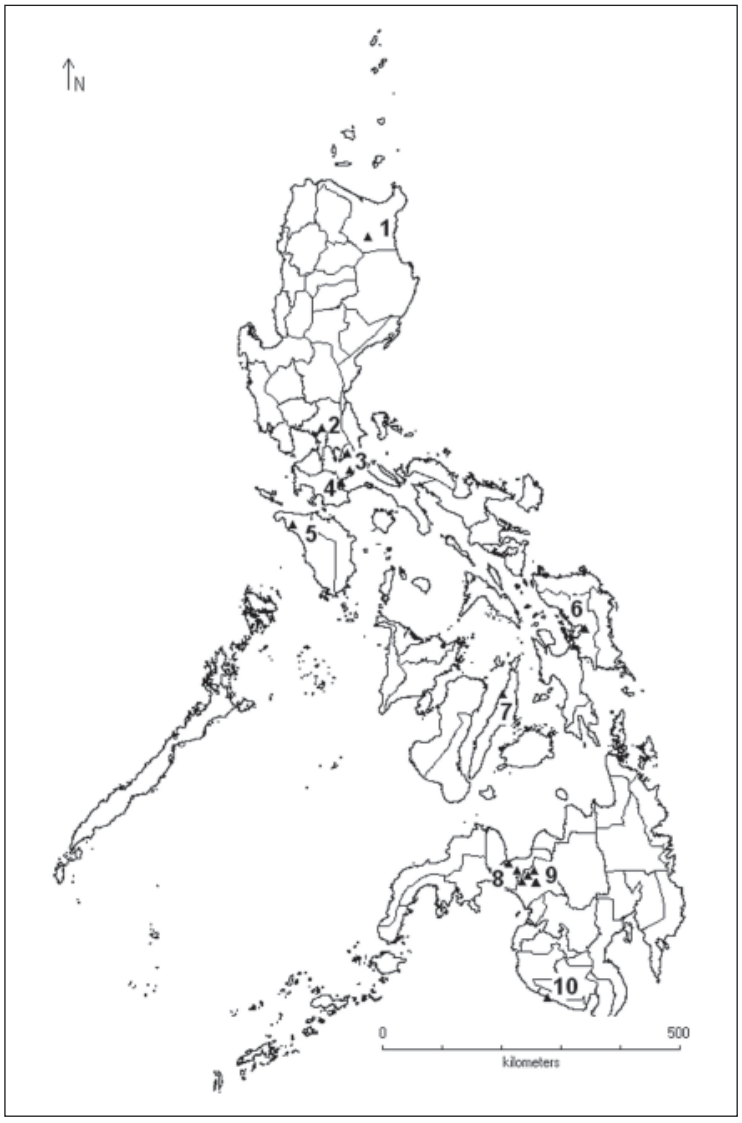

Figure 1. Map of the Philippines, showing occurrence or collection sites of *An. vagus*, in triangle symbol. Province identification: (1) Cagayan, (2) Bulacan, (3) Quezon), (4) Laguna, (5) Mindoro Occidental, (6) Samar, (7) Cebu, (8) Lanao del Norte, (9) Lanao del Sur, (10) South Cotabato

one of the 19 *Anopheles* species that co-transmit *Plasmodium* and *W. bancrofti* in Asia. Although Japanese encephalitis (JE) virus was found in 1 out of 42 pools of *An. vagus* from Lombok Island, Indonesia (Olson *et al.*, 1985), no further isolates have been reported. All of these findings regarding the vector roles of *An. vagus* infer that this species should not be forgotten or disregarded as a potential vector when outbreaks of human disease occur in unusual scenarios where well recognized vectors and usual hosts (cows and water buffalos) are uncommon or absent.

Anopheles vagus should not be considered a primary vector of malaria due to its skewed preference for feeding on bovids, however, evidence has accrued over the last 3-4 decades showing it can function as a secondary vector in the absence of these preferred hosts. Because of the preferred larval habitats of this species and its ability to survive in small pools of muddy and organically polluted water in full sunlight it will continue to be a common species in Southeast Asia. In a future with increasing human populations, warming temperatures, increasing ocean levels, loss of forests and subsequent increases in larval habitats for *An. vagus*, less reliance on single family farming that requires bovids and more reliance on commercialized processed and preserved foods, humans will undoubtedly be forced into more concentrated environs that are likely to promote the role of *An. vagus* as a secondary vector of human pathogens.

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